The magnitude of airway remodelling is not altered by distinct allergic inflammatory responses in BALB/c vs C57BL/6 mice but matrix composition differs

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Running title:
Allergic airway inflammation and remodelling in mice

Keywords:

Allergic airway inflammation; type 2 cytokines; type 17 cytokines; extracellular matrix; airway remodelling; collagen; mouse strain differences

Abstract

Allergic airway inflammation is heterogenous with variability in immune phenotypes observed across asthmatic patients. Inflammation has been thought to directly contribute to airway remodelling in asthma, but clinical data suggests that neutralising type 2 cytokines does not necessarily alter disease pathogenesis. Here, we utilised C57BL/6 and BALB/c mice to investigate the development of allergic airway inflammation and remodelling. Exposure to an allergen cocktail for up to 8 weeks led to type 2 and type 17 inflammation, characterized by airway eosinophilia and neutrophilia and increased expression of chitinase-like proteins in both C75BL/6 and BALB/c mice. However, BALB/c mice developed much greater inflammatory responses than C57BL/6 mice, effects possibly explained by a failure to induce pathways that regulate and maintain T cell activation in C57BL/6 mice, as shown by whole lung RNA transcript analysis. Allergen administration resulted in a similar degree of airway remodelling between mouse strains but with differences in collagen subtype composition. Increased collagen III was observed around the airways of C57BL/6 but not BALB/c mice while allergen-induced loss of basement membrane collagen IV was only observed in BALB/c mice. This study highlights a model of type 2/type 17 airway inflammation in mice whereby development of airway remodelling can occur in both BALB/c and C57BL/6 mice despite differences in immune response dynamics between strains. Importantly, compositional changes in the ECM between genetic strains of mice may help us better understand the relationships between lung function, remodelling and airway inflammation.

Introduction

Asthma is a global health problem with increasing prevalence, currently affecting over 300 million people.[1] Of note, the term "asthma" encompasses a range of disease phenotypes. Progress in understanding heterogeneity of airway inflammation has led to defined asthma endotypes[2,3] often characterized by the presence or absence of type 2 eosinophilic inflammation and/or type 17 neutrophilic inflammation.[4] A clarified definition of such inflammatory phenotypes in asthma has led to development of innovative therapies directed at modulating specific inflammatory pathways, in particular type 2 inflammation.[5] Whilst the use of biologicals targeting IgE or either type 2 cytokines Interleukin (IL)-4, IL-5, IL-13 or their cognate receptors (IL-4Rα, IL-5R, IL-13Rα1) have been effective at reducing disease exacerbations in allergic asthmatics, these therapies are often insufficient to improve underlying disease pathogenesis.[6-8] Furthermore, clinical trials with antibodies targeting IL-17 signalling have shown no benefit[9], despite a strong association of severe asthma with type 17 neutrophilic inflammation.[10] To achieve progress in treating asthma we need a more comprehensive understanding of pathology beyond viewing inflammation as the main instigator of disease.

Along with airway inflammation, asthma is characterized by airway hyper-responsiveness (AHR) and airway remodelling, a process of changes to the composition, content and organisation of cells and extracellular matrix in the lung. Although tissue remodelling is a critical process during development and tissue repair[11], it is also a pathogenic response in diseases like asthma, and undoubtedly impacts on lung function.[12] Comprehensive research using a combination of mouse models and human studies typically attribute the development of remodelling to chronic airway inflammation.[13-17] However, this view conflicts with emerging data that shows remodelling can occur as a primary event prior to inflammation.[18-20] Glucocorticoid steroids can generally improve lung function but do not alter airway remodelling.[21] Alternatively, drugs that successfully target specific inflammatory pathways fail to improve lung function[7,8] presumably because they do not affect airway remodelling. Overall, the links between inflammation, remodelling and lung function are still unclear

and thus warrant investigation. Airway remodelling is a complex disease process, difficult to study in patients especially in the context of understanding multiple components that may influence development of individual remodelling processes over time. Therefore, there is a crucial need for animal models that reflect asthma disease processes with a focus on studying the development of stable and irreversible airway remodelling.

Genetic differences between inbred mouse strains are well known to strongly affect both airway inflammation[22-25] and AHR.[26] For instance, C57BL/6 mice are known to have a high airway resistance in response to methacholine challenge independent of allergic inflammation[22], whereas BALB/c mice generally exhibit greater airway reactivity in response to allergens.[26] Therefore, studies examining airway pathology in different mouse strains can provide a basis to explore the relationships between immune cell dynamics in relation to changes in airway remodelling and lung function. In this study we utilised a model of chronic allergic airway inflammation that shares features of disease common to severe asthma in people, including mixed type 2 and type 17 airway inflammation, steroid resistant neutrophilia and AHR independent of type 2 cytokines[27,28] to investigate inflammation and remodelling parameters. Together our results highlight mouse strain dependent differences in type 2 and type 17 inflammation that do not seem to alter the development of remodelling but may impact on deposition of specific collagen subtypes.

Results

Chronic allergen-induced immune response dynamics differ between C57BL/6 and BALB/c mouse strains.

Aspects of allergen-induced airway inflammation have been extensively studied in mouse models, largely in the context of acute Th2-mediated immune responses.[29,30] Here, using a model of mixed type 2 and type 17 inflammation, we characterised immune cell dynamics in two common in-bred mouse strains, C57BL/6 and BALB/c, strains known to respond differently in models of airway inflammation and hyper-responsiveness.[26,31,32] Mice were exposed to a multi-allergen cocktail (House Dust Mite, Ragweed and *Aspergillus fumigatus*; DRA) for 4 or 8 weeks (**Figure 1a**) and inflammation assessed. All mice exposed to DRA had a mixed neutrophilic/eosinophilic inflammation, although neutrophilia was not evident in C57BL/6 mice until week 8 (**Figure 1b**). Neutrophils were still present in the BAL 5 days after the last allergen administration (**Figure 1a, b**), even though their numbers were considerably lower compared to eosinophils (**Figure 1b**). Analysis of T lymphocytes and related populations in the lungs, revealed relatively similar proportions of allergen-induced immune cell accumulation between mouse strains, with gradual increases in ICOS$^+$ innate lymphoid cells (ILCs) (**Figure 1c**). BALB/c mice generally had a greater ratio of CD4+ to CD8+ T cells compared to C57BL/6 mice reflected in higher total lung CD4+ T cell numbers (**Supplementary figure 1**), but this ratio did not change due to allergen exposure (**Figure 1c**). In addition, innate populations of γδT cells and ICOS$^+$ ILCs were more predominant in BALB/c mice compared to C57BL/6 mice with clear increases in cell numbers in BALB/c mice following allergen exposure at either week 4 or 8 (**Figure 1c and supplementary figure 1**). Despite the increased ILC numbers (**Supplementary figure 1**), CD4$^+$ T cells and γδT cells appeared to be the major cytokine producing lymphocyte populations in the lungs of all allergic mice. Exposure to DRA resulted in both IL-17A and type 2 inflammatory responses in the lung, with an increased proportion of IL-4 and IL-17A expressing CD4$^+$ T cells in both C57BL/6 and BALB/c mice (**Figure 2a**). Interestingly, the numbers of IL-4$^+$ CD4$^+$ T cells were reduced from week 4 to week 8 in BALB/c mice (**Figure 2b**), also corresponding to a reduction in eosinophils (**Figure 1b**). Nonetheless, there were enhanced numbers of IL-17A$^+$ TCRγδ$^+$ T cells and IL-17A$^+$ CD4$^+$ T as well as IL-4$^+$ CD4$^+$ T cells on week 4 in BALB/c compared to C57BL/6 mice (**Figure**

2b). Of note, TCRγδ$^+$ and CD4$^+$ T cells contributed equally to the pool of IL-17A$^+$ cells in allergic BALB/c mice, whereas CD4$^+$ T cells were the main IL-17A$^+$ population in C57BL/6 mice (**Figure 2b**).

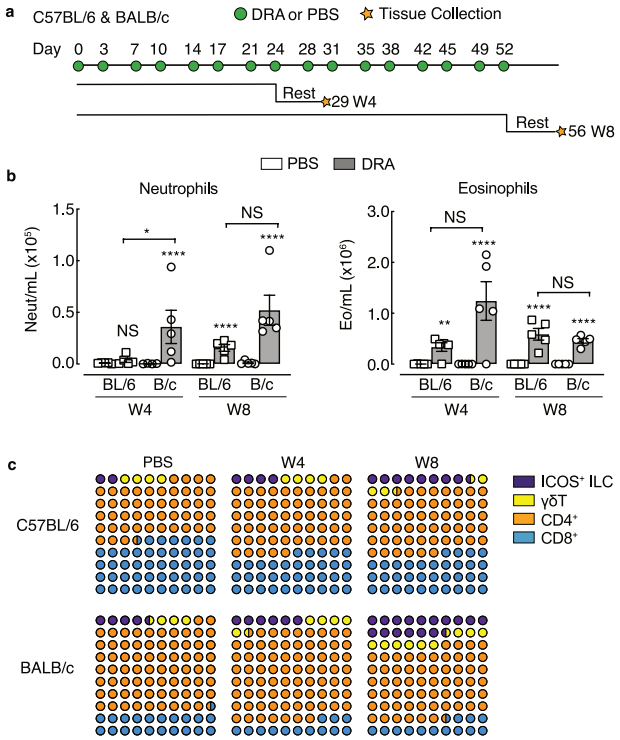

Figure 1: Chronic exposure to DRA allergens induce neutrophil and eosinophil airway inflammation

a) Schematic showing allergic airway inflammation model highlighting the timing of DRA or PBS intranasal administration into C57BL/6 or BALB/c mice. Cells were collected for flow cytometry analysis 5 days after the last administration of PBS or DRA (rest) at either 4 or 8 weeks. **b**) Numbers of neutrophils and eosinophils in the BAL of C57BL/6 or BALB/c mice administered PBS or DRA for 4 or 8 weeks. **c**) Plot showing the average proportions of different T cells and ILCs in the lungs of C57BL/6 or BALB/c mice administered PBS or DRA for 4 or 8 weeks. Data are representative of 2 experiments. Data is plotted as mean ± sem with points representing individual animals (**b**). Data in **b** was analysed by ANOVA with Tukey's multiple comparison test with significance level showing comparisons between either PBS animals within each strain and each time point or C57BL/6 to BALB/c mice as indicated on the graph. NS not significant, *P<0.05, ****P<0.0001.

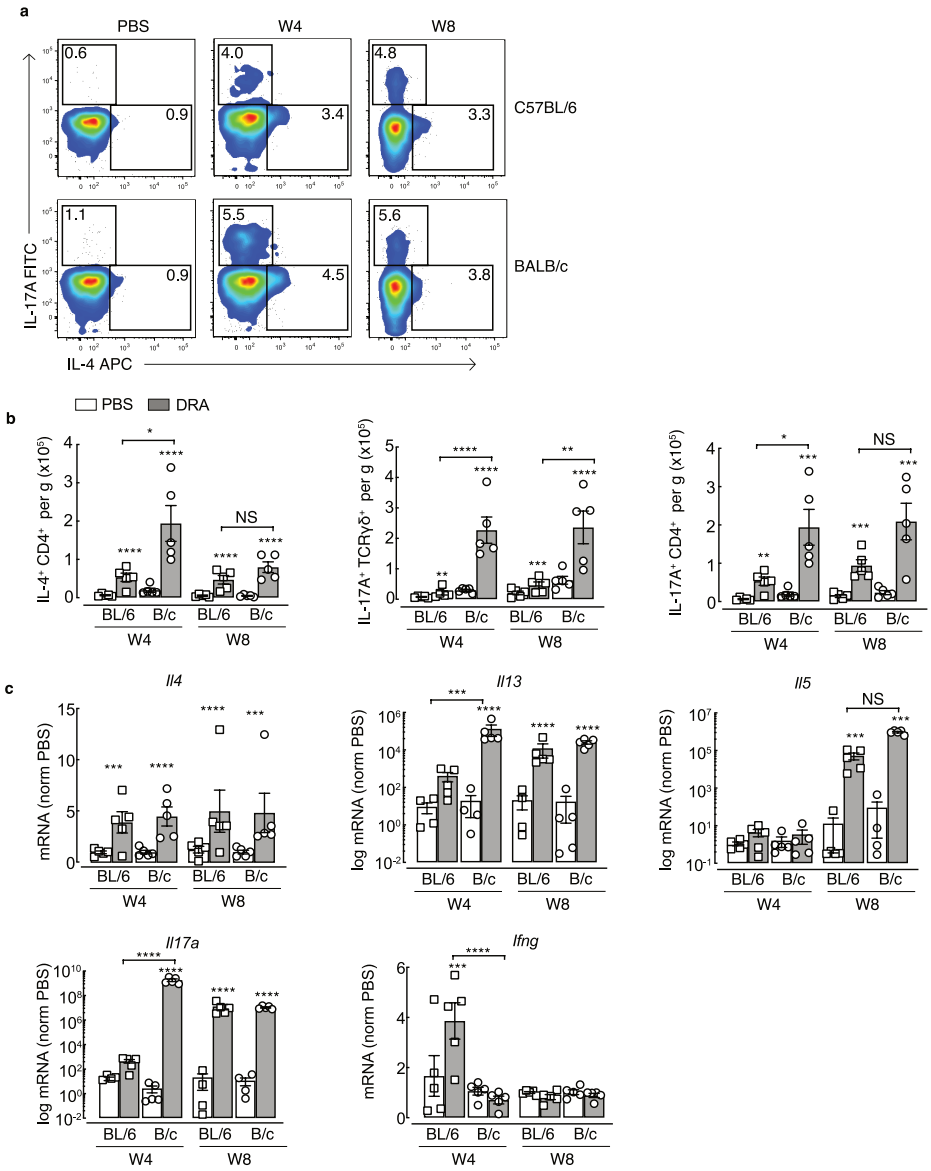

Figure 2: Chronic exposure to DRA allergens induce mixed Th2/Th17 airway inflammation
a) Whole lung single cell suspensions were stained for flow cytometry. Live, single TCRγδ$^-$TCRβ$^+$ CD8$^-$CD4$^+$ cells were gated on and representative intracellular staining plots of IL-4$^+$ and IL-17A$^+$ CD4$^+$ T cells in the lungs of C57BL/6 or BALB/c mice administered PBS or DRA intranasally two times a week for up to 8 weeks. Cells were analysed by flow cytometry 5 days after the last instillation of allergen. Single cell lung suspensions were stimulated with PMA/ionomycin prior to analysis by flow cytometry. Numbers indicate the percentage of cytokine positive CD4$^+$ T cells within each gate. **b**) Absolute numbers of IL-17A$^+$ TCRγδ$^+$ or IL-17A$^+$ or IL-4$^+$ CD4$^+$ T cells in the lungs of mice as in **a**. **c**) mRNA expression of *Il4*, *Il13*, *Il5*, *Il17a* and *Ifng* in whole lungs of mice treated as in **a.** mRNA were normalised to levels found in PBS C57BL/6 or BALB/c mice at each time point and are relative to geometric mean of housekeeping genes *Gapdh*, *Rpl13a* and *Rn45s*. Data are representative of 2 experiments. Data is plotted as mean ± sem with points representing individual animals. Data was analysed by ANOVA with Tukey's multiple comparison test with significance level showing comparisons between either PBS animals within each strain and each time point or C57BL/6 to BALB/c mice as indicated on the graph. NS not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

185 Expression of key cytokines in whole lung RNA also revealed an increase in type 2 genes
186 (*Il4*, *Il5*, *Il13*) and *Il17a* upon DRA treatment with exaggerated *Il13* and *Il17a* expression
187 at week 4 in allergic BALB/c compared to C57BL/6 mice (**Figure 2c**). Whilst we saw no
188 evidence of increased IFNγ^{+} T cells or ILCs (data not shown) in the lungs of mice
189 following allergen administration, there was a transient increase in *Ifng* expression in the
190 lungs of C57BL/6 mice but not BALB/c (**Figure 2c**). Overall, although both mouse strains
191 developed allergen-induced airway inflammation, the degree of inflammation and
192 eosinophilic and neutrophilic responses were higher in BALB/c compared to C57BL/6
193 mice.
194
195 **Chitinase-like proteins are abundantly expressed in the lungs during type 2 and type 17**
196 **allergic airway inflammation**
197 Chitinase-like proteins (CLPs) are molecules strongly associated with severe asthma,
198 neutrophilia and IL-17A.[33-36] Following exposure to DRA allergen, mRNA expression of
199 murine CLPs *Chil1*, *Chil3* and *Chil4* were upregulated in both BALB/c and C57BL/6 mice
200 compared to PBS controls (**Figure 3a**). However, *Chil1* mRNA expression was less in
201 C57BL/6 compared to BALB/c mice, albeit this did not reach statistical significance in
202 this dataset (*P* 0.07 DRA C57BL/6 versus DRA BALB/c mice at week 4 or 8). Additionally,
203 no significant increase in *Chil1* mRNA was detected in whole lung tissue of C57BL/6
204 mice after 4 weeks of allergen exposure as compared to PBS controls (**Figure 3a**) despite
205 significant increases in secreted BRP-39 protein levels in the BAL (**Figure 3b**). *Chil3* and
206 *Chil4* were significantly increased in both mouse strains at week 4, and expression levels
207 did not change upon further allergen exposure (**Figure 3a**), findings that were supported
208 by measurement of Ym1 secreted protein in the BAL (**Figure 3b**). As there were no
209 commercially available reagents to measure Ym2 protein levels, we developed a Ym2
210 specific antibody to examine Ym2 expression in the lungs (**Supplementary figure 2**). By
211 western blot, neither Ym1 nor Ym2 was detected in mice administered PBS, but both
212 Ym1 and Ym2 greatly increased following allergen exposure (**Figure 3c**). CLPs can be
213 readily detected in the serum, and serum levels of YKL-40 in humans has been proposed
214 as a biomarker for disease severity and is associated with reduced lung function in
215 several pulmonary pathologies.[37-39]

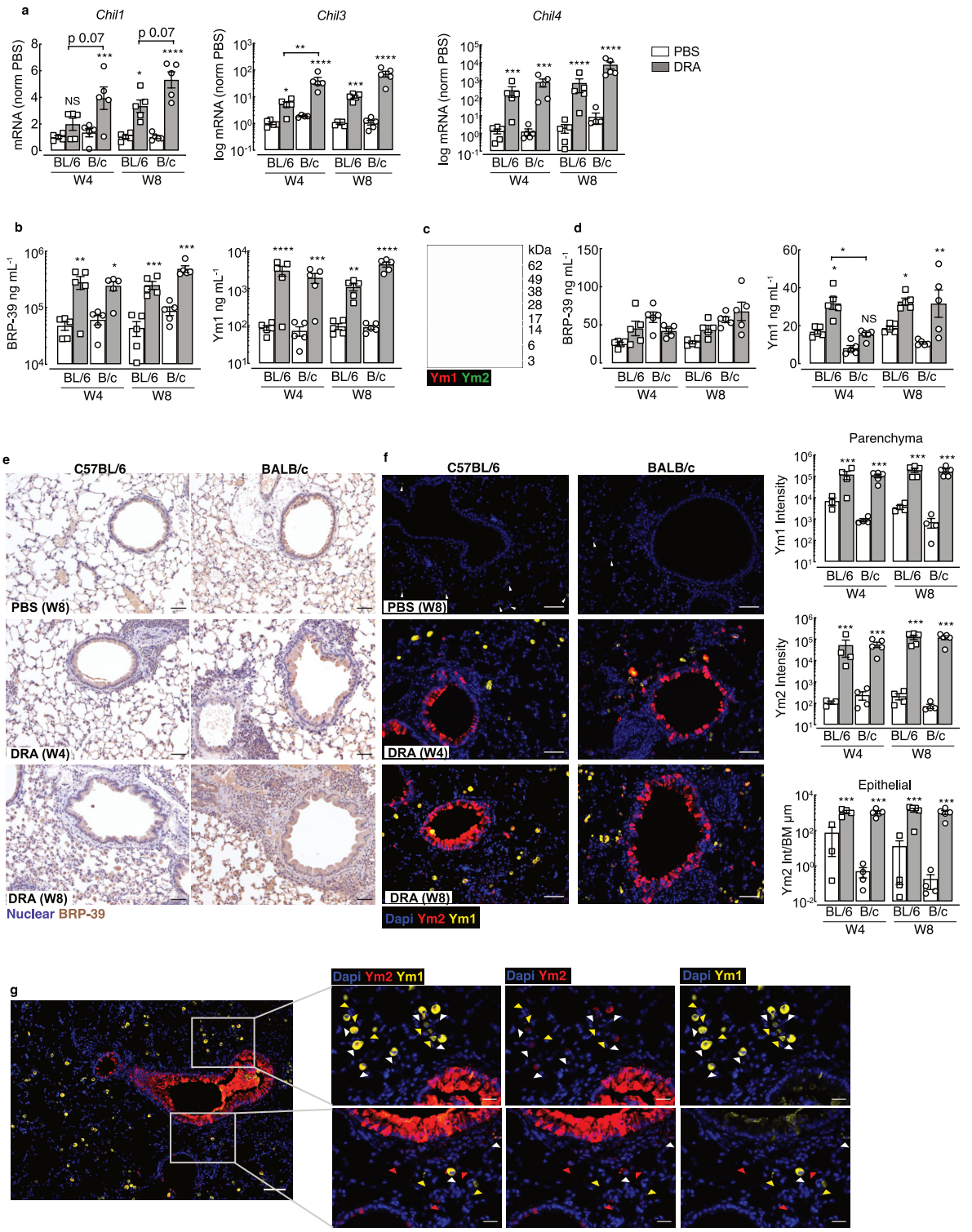

Figure 3: Chitinase-like proteins are abundantly expressed during chronic allergic airway inflammation

a) mRNA expression of CLPs *Chil1*, *Chil3* and *Chil4* in whole lungs of C57BL/6 or BALB/c mice exposed intranasally to PBS or DRA for 4 or 8 weeks. Lungs were collected 5 days after the last PBS/DRA administration. mRNA were normalised to levels found in PBS C57BL/6 or BALB/c mice at each time point and are relative to the geometric mean of housekeeping genes *Gapdh*, *Rpl13a* and *Rn45s*. *Chil3* and *Chil4* is depicted as log mRNA levels. **b**) Concentration of Ym1

and BRP-39 protein measured by ELISA in the BAL of C57BL/6 or BALB/c mice treated as in **a**. **c**) Western blot analysis of Ym1 (red) and Ym2 (green) levels in the BAL from C57BL/6 mice administered with PBS or DRA for 8 weeks, with BAL taken 5 days after the last DRA/PBS administration. **e**) Concentration of Ym1 and BRP-39 protein measured by ELISA in serum of C57BL/6 or BALB/c mice treated as in **a**. **e**) Microscopy images of immunohistochemical staining of BRP-39 (brown) in lung sections from C57BL/6 and BALB/c mice treated with either PBS for 8 weeks, or DRA for 4 or 8 weeks. Cell nuclei counterstained with haematoxylin (purple); scale bar 50 µm. **f**) Microscopy images of lungs sections of mice as in **a** stained with DNA-binding dye (DAPI) blue; Ym1 (yellow) and Ym2 (red). Scale bar; 50 µm. Images are representative of n=5 mice per group. Quantification of antibody positive staining intensity from stained sections. Ym1 and Ym2 intensity in lung parenchyma areas and Ym2 intensity in airway epithelial cells normalised to length of airway basement membrane. **g**) Microscopy images of immunofluorescent staining for Ym1 (yellow) and Ym2 (red) in lung sections for mice as in **f**. Images show areas where co-staining in airway epithelial or parenchyma cells is evident. Triangles superimposed onto images show $Ym1^{+}Ym2^{-}$ cells (yellow), $Ym1^{-}Ym2^{+}$ cells (red) or $Ym1^{+}Ym2^{+}$ cells (white). Centre image scale bar, 100 µm; outer images scale bar, 50 µm. Datapoints depict individual animals with bars representing mean and sem (**a, b, d, f**). Data are representative of 2 experiments. Data were analysed by ANOVA with Tukey's multiple comparison test with significance level showing comparisons between either PBS animals within each strain and each time point or C57BL/6 to BALB/c mice as indicated on the graph. NS not significant; *P<0.05, ** P<0.01, ***P<0.001, ****P<0.0001.

Although BRP-39 is a genetic ortholog of YKL-40, the level of BRP-39 in the blood was not significantly altered following allergic-inflammation in this model (**Figure 3d**). However, increased serum Ym1 was detectable in allergic mice of both strains (**Figure 3d**). To determine whether localisation of the three CLPs differed between strains of mice following allergen exposure, we examined immunostained lung sections. BRP-39 was already expressed in macrophages and epithelial cells in the steady state, but the intensity and number of positive cells increased further following allergen administration and the increase was particularly evident in BALB/c mice (**Figure 3e**). Corresponding to secreted levels in the BAL (**Figure 3c**), expression of Ym2 was absent in the lungs of PBS mice, while numerous $Ym1^{+}$ cells, likely alveolar macrophages could be detected (**Figure 3f**).[40] The expression of Ym1 and Ym2 dramatically increased in the lungs of allergic BALB/c or C57BL/6 mice and the level of expression reached its maximum expression at 4 weeks post DRA treatment (**Figure 3f**). Interestingly, Ym1 and Ym2 appear to have a fairly distinct expression pattern in the lung, with Ym1 largely restricted to myeloid cells and Ym2 largely expressed by epithelial cells, and very few cells that co-stained for Ym1 and Ym2 (**Figure 3f, g**). Overall, we observed modest increases in BRP-39 levels in

265 allergic animals, but strongly enhanced Ym1 and Ym2 expression in the lungs of both
266 allergic C57BL/6 and BALB/c mice. For the first time we show distinct expression of Ym2
267 in the lungs compared to Ym1, despite their protein sequence being ~96% homologous.
268
269
270 **Allergen-induced immune pathways are fundamentally different between C57/BL6 and**
271 **BALB/c mouse strains**
272 C57BL/6 and BALB/c mice both developed neutrophilic and eosinophilic airway
273 inflammation in response to chronic allergen administration, despite a greater magnitude
274 of both type 2 and IL-17A cytokine responses in BALB/c mice. Therefore, to more broadly
275 characterise the differences in immune response between mouse strains, we performed
276 differential gene expression analysis of whole lung RNA after 8 weeks of allergen or PBS
277 administration using the NanoString nCounter Myeloid Innate Immunity Panel
278 (NanoString, Amersham, UK). Principal component analysis (PCA) demonstrated a clear
279 separation in gene signatures not only from exposure to DRA versus PBS, Principal
280 Component (PC) 1, but also mouse strain, explained by PC2 (**Figure 4a**). Investigation of
281 the genes that were significantly altered in the DRA model showed that numerous genes
282 were induced (e.g. type 2 effector molecules *Retnla* and *Arg1*) or inhibited (e.g. basement
283 membrane collagens, *Col4a1*, *Col4a1*) to an equivalent degree in both strains
284 (**Supplementary figure 3a**). Hierarchical clustering also separated a considerable number
285 of genes that were regulated in the same way across the strains, but to a much higher
286 degree in one mouse strain over the other (**Figure 4b**) or expression of genes that were
287 fundamentally different between strains (**Supplementary figure 3b**). As predicted from
288 the allergic cytokine responses and alterations in immune cell infiltration into the lung
289 (**Fig 1 & 2**), it was not surprising that type 2 related genes such as *Il13*, *Fcer2a*, *Csf2, Ccl2*,
290 *Ccl11* were more highly upregulated in whole lung tissue from BALB/c compared to
291 C57BL/6 mice (**Figure 4b**). However, interestingly factors known to play an important
292 role in leukocyte adhesion (*Itgb2*, *Itgb7*, *Selplg*) were upregulated in allergic C57BL/6
293 mice but not BALB/c mice (**Figure 4b**), despite an apparent slower rate of inflammatory
294 cell accumulation in C57BL/6 compared to BALB/c mice (**Figure 1b and supplementary**
295 **figure 1**).
296

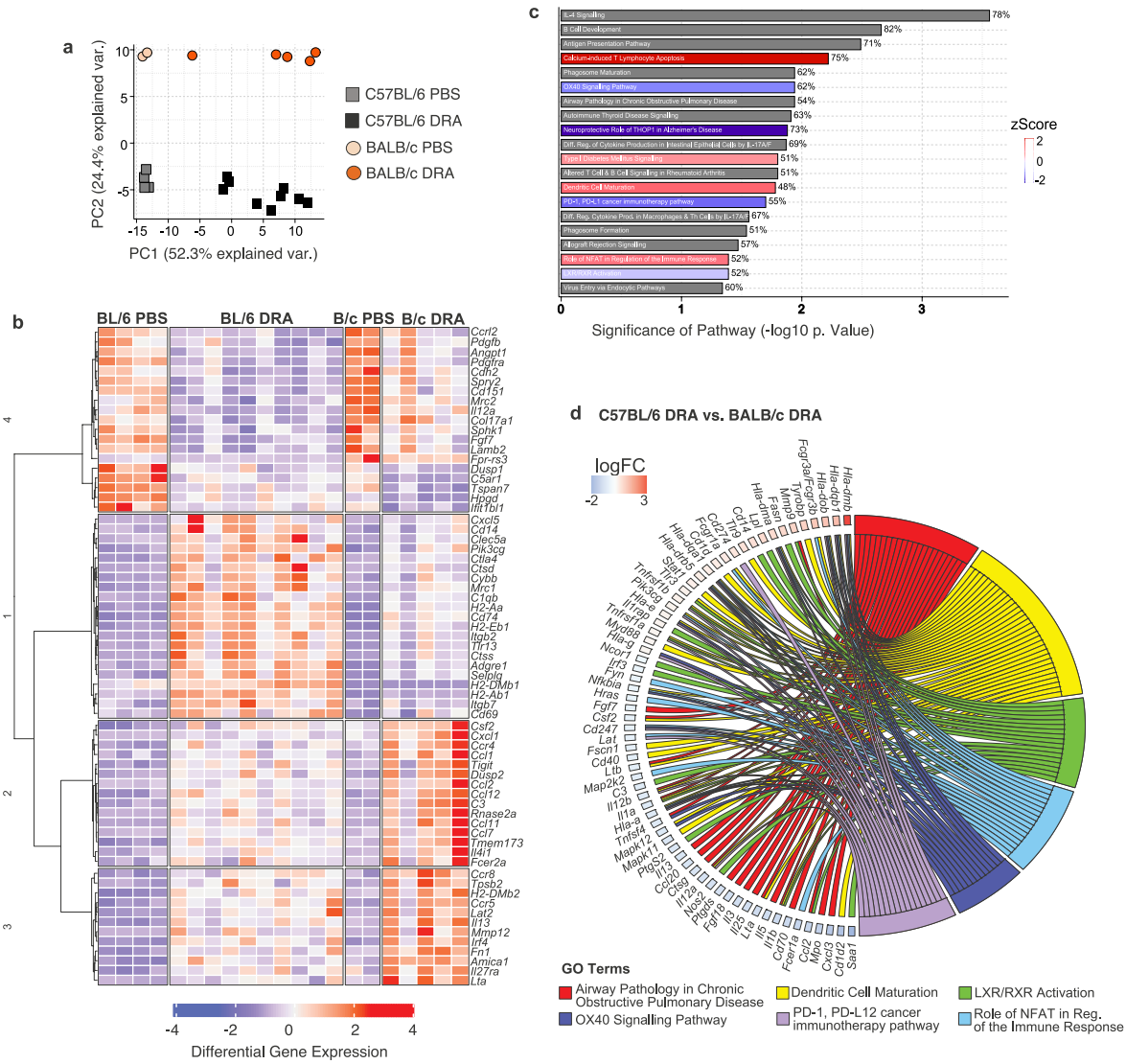

Figure 4: C57BL/6 and BALB/c allergic mice have fundamental differences in immune gene signatures

Whole lung RNA from C57BL/6 and BALB/c mice administered with either PBS or DRA for 8 weeks were analysed using NanoString Myeloid Panel v2. **a**) PCA of expressed genes from C57BL/6 and BALB/c. **b**) Unsupervised, hierarchically clustered heatmap of genes that were significantly regulated in C57BL/6 and BALB/c allergic compared to PBS mice, but also differential regulated between the treated strains. **c**) Differentially expressed genes were visualised with Ingenuity Pathway Analysis tool and top 20 canonical pathways shown for C57BL/6 versus BALB/c mice. Red or blue indicates pathways upregulated or downregulated (respectively) in C57BL/6 compared BALB/c allergic mice. Grey indicates pathways that are significantly regulated but not in a particular direction. Percentage at end of the bar equates to the number of molecules detected compared to the total number of molecules within the canonical pathway. **d**) Chord diagram shows specific genes up or down regulated (colour indicating log fold change) within Go Term that were found to be significantly regulated in C57BL/6 allergic mice compared to BALB/c allergic mice. Transcriptomic analysis was performed on one experiment that was representative of 2 individual experiments.

316 Analysis of common properties within a signalling pathway (canonical pathway) showed
317 enrichment of various pathways in C57BL/6 compared to BALB/c mice (**Figure 4c**).
318 Pathways including 'IL-4 signalling' and 'airway pathology in COPD' were significantly
319 different across mouse strains (**Figure 4c**). Whether these pathways were activated or
320 inhibited in C57BL/6 compared to BALB/c mice could not be clearly defined by the
321 analysis (as denoted by the grey bar; **Figure 4c**). However, the specific genes that
322 contributed to the z scores (**Figure 4c**) were also examined (**Figure 4d**). For example, a
323 downregulation of both type 2 cytokines *Il5*, *Il13* and the type 2 inducing cytokine *Il25*
324 in addition to reduced expression of pro-inflammatory cytokines *Il1a*, *Il1b*, *Il12a* and
325 *Il12b* indicates that genes characteristic of the "airway pathology in COPD' pathway
326 (**Figure 4d**) were reduced in allergic C57BL/6 mice relative to BALB/c mice. Interestingly,
327 both 'OX40 signalling' and 'PD-1, PD-L1 signalling', pathways involved in maintenance
328 and regulation of T cell responses, were downregulated in allergic C57BL/6 compared
329 to BALB/c mice (**Figure 4d**). These and other changes in canonical pathways involved in
330 DC-T cell stimulation possibly explain reduced cytokine production in C57BL/6 mice
331 (**Figure 2**). In addition, LXR/RXR activation, which maintains cholesterol homeostasis but
332 is also known to be anti-fibrotic and anti-inflammatory[41], was downregulated in C57BL/6
333 mice (**Figure 4c, d**). Overall, analysis of gene regulation at chronic allergic inflammatory
334 time points revealed differences in gene signatures between mouse strains that may
335 explain reduced immune responses in C57BL/6 compared to BALB/c mice.
336
337 **Airway remodelling develops in both C57BL/6 and BALB/c mice despite different**
338 **allergic inflammation dynamics and immune signatures**
339 The relationship between inflammation and airway remodelling in asthma is still
340 controversial (reviewed by Saglani & Lloyd[42], Boulet[43], Guida & Riccio[44]). Some features
341 of remodelling may occur in parallel or even prior to excessive inflammation[18-20]
342 although difficult to test in the clinical setting. Considering different immune cell
343 dynamics between BALB/c and C57BL/6 mice (**Figure 1 - 4**), we sought to determine
344 whether features of airway remodelling also varied between mouse strains. Goblet cell
345 hyperplasia is a key feature of remodelling in asthma and contributes to excessive airway
346 mucus secretion.
347

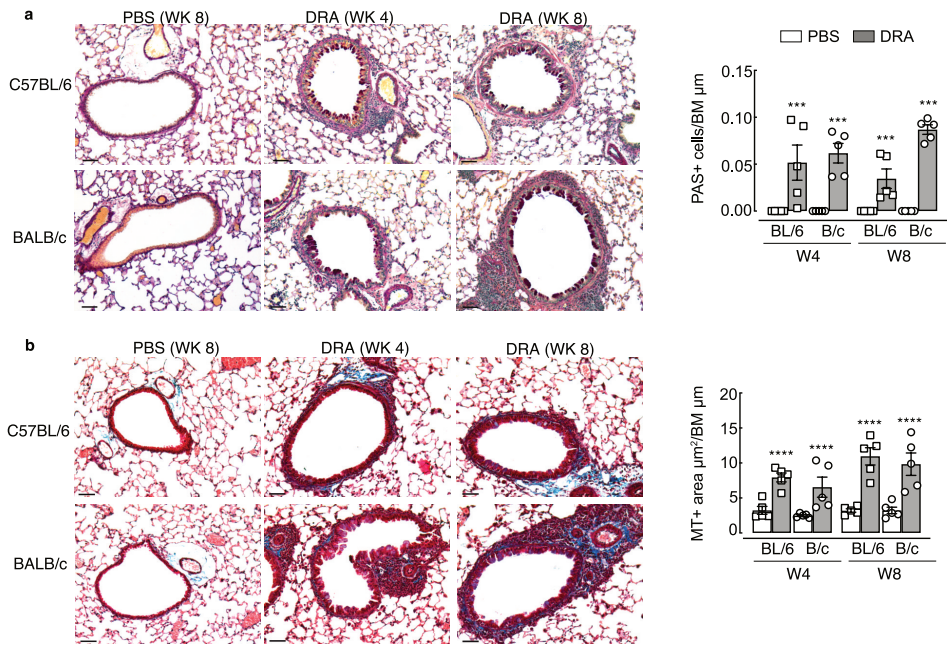

Figure 5: Goblet cell numbers and total collagen increases around the airways following exposure to DRA allergens.
C57BL/6 or BALB/c mice were intranasally administered PBS or DRA twice a week up to 8 weeks, and lungs collected for histological analysis 5 days after the last PBS or DRA at weeks 4 and 8. **a**) Microscopy images of lung sections stained for PAS. Airways show PAS$^+$ cells (purple) within the epithelium. Graph shows quantification of numbers of PAS$^+$ cells per length of basement membrane. **b**) Microscopy images of lung sections stained for Masson's trichrome from C57BL/6 or BALB/c mice. Airways show accumulation of collagen (blue) below the basement membrane. Graph shows quantification of the area of Masson's trichrome positive staining around the airways normalised to basement membrane length. All images are representative of n=5 mice; scale bar equals 50 µm. Datapoints depict individual animals with bars representing mean and sem. Data are representative of 2 experiments and were analysed by ANOVA with Tukey's multiple comparison test and significance level shown relative to PBS animals within each strain and each time point. ***P<0.001, ****P<0.0001.

Equivalent increases in periodic acid schiff (PAS) positive cells, indicative of goblet cells, were observed in both C57BL/6 and BALB/c mice at weeks 4 and 8 (**Figure 5a**). Airway remodelling in asthmatic patients is also characterized by thickening of the basement membrane and deposition of sub-epithelial extracellular matrix proteins. Following DRA allergen exposure, increased collagen deposition, measured by Masson's Trichrome stain, was also evident around the airways of both mouse strains (**Figure 5b**). Specific immunostaining for components of the ECM (**Figure 6a, c**) previously described to be regulated in asthma[45-48], supported increases in total airway collagen following allergen exposure (**Figure 5b**). However, fundamental differences in collagen expression between mouse strains were also evident (**Figure 6a, b**).

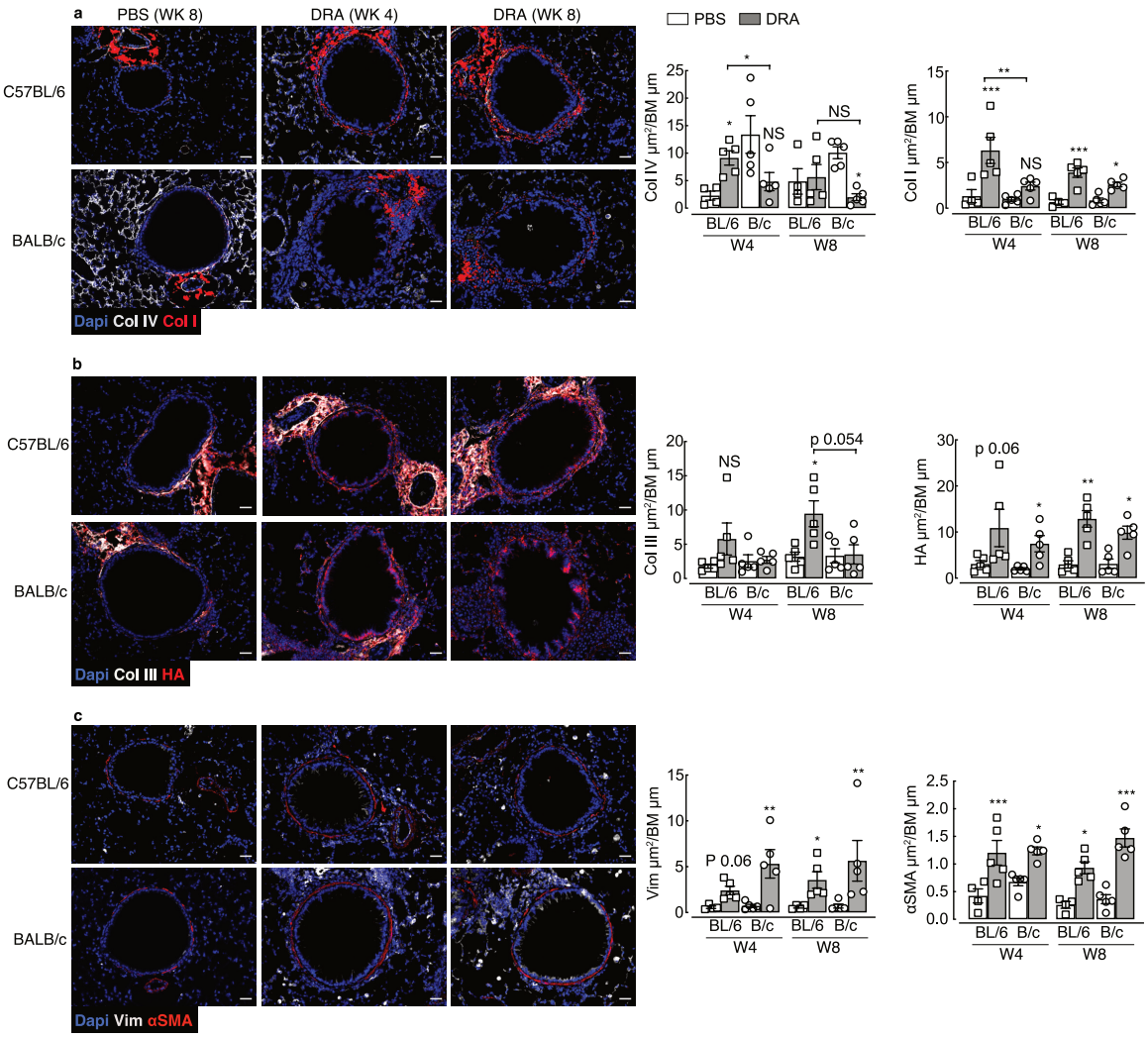

Figure 6: Changes to the ECM and muscle mass around the airway occur following exposure to DRA allergens.
C57BL/6 or BALB/c mice were intranasally administered PBS or DRA twice a week up to 8 weeks, and lungs collected for immuno-staining 5 days after the last PBS or DRA at weeks 4 and 8. **a-c**) Microscopy images of lung sections from C57BL/6 or BALB/c mice stained with DNA-binding dye (DAPI) blue; (**a**) Col IV white; Col I red; (**b**) Col III, white; hyaluronan (HA) binding protein, red; (**c**) vimentin (Vim) white; alpha smooth muscle actin (αSMA) red. Scale bar, 30 µm. Images are representative of n=5 mice. Antibody positive staining area was quantified around the airway and normalised to basement membrane length and values are depicted in **a-c**. Datapoints depict individual animals with bars representing mean and sem. Data are representative of 2 experiments and were analysed by ANOVA with Tukey's multiple comparison test and significance level showing comparisons between either PBS animals within each strain and each time point or C57BL/6 to BALB/c mice as indicated on the graph. *P<0.05, ** P<0.01, ***P<0.001.

Basement membrane protein collagen IV was highly expressed in the steady-state around the airways and alveoli of BALB/c mice compared to C57BL/6 (**Figure 6a**). Upon allergen

395 administration, collagen IV expression decreased over time in BALB/c mice, whereas
396 levels transiently increased in C57BL/6 mice. Additionally, a greater and more rapid
397 increase in airway collagen I in allergic C57BL/6 compared to BALB/c mice was observed
398 (**Figure 6a**) and similarly, accumulation of airway collagen III was significantly increased
399 only in allergic C57BL/6 mice (**Figure 6b**). In contrast, expression of a major
400 glycosaminoglycan component of the ECM, hyaluronan (HA), was increased in response
401 to allergen exposure independently of mouse strain (**Figure 6b**). Changes to collagen
402 composition around the airways of allergic mice was accompanied by an increase in the
403 number of vimentin positive cells (**Figure 6c**), potentially indicating an increase in
404 matrix-secreting fibroblasts.[49] Additionally, airway muscle mass was also examined and
405 revealed increases in allergic mice regardless of mouse strain (**Figure 6c**). Together, these
406 results demonstrate features of remodelling such as goblet cell hyperplasia, increased
407 smooth muscle mass and ECM changes occur in both C57BL/6 and BALB/c mice.
408 However, differences in deposition of specific collagen subtypes exists between mouse
409 strains.
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411
412

413 ## Discussion

414 IL-17 and neutrophilia are often associated with severe asthma.[10] Despite this, models of
415 allergic airway inflammation still largely focus on studying the regulation of allergen
416 induced type 2 immune responses, utilising BALB/c mice that generally show a strongly
417 skewed type 2 inflammatory response.[26] Here, we utilised a model of allergic airway
418 inflammation in which neutrophilia and IL-17 are dominant features, with inflammation
419 resistant to steroid intervention [28] and AHR unaffected by neutralisation of IL-5 or IL-13
420 cytokines.[27] As expected BALB/c mice developed rapid and prominent airway
421 inflammation that was skewed toward type 2 responses, but also greater IL-17
422 production, particularly by γδ T cells. However, type 2 inflammation was reduced from
423 week 4 to week 8 perhaps reflecting the emergence of a tolerogenic response to allergens
424 in BALB/c mice.[25] C57BL/6 mice still responded to allergens, but Th2 and IL-17A
425 responses developed at a slower rate compared to BALB/c mice. Delayed type 2 cytokine

426 expression is potentially explained by an early but transient spike in IFNγ expression
427 only observed in C57BL/6 mice. Interestingly, increased IL-17A expression in C57BL/6
428 mice between weeks 4 and 8 coincided with a reduction in IFNγ levels in allergic mice,
429 which we have shown previously to be an important factor that allows the development
430 of a pulmonary type 2 immune response.[50] Additionally, IL-10 derived from T cells has
431 been shown to signal via alveolar macrophages leading to suppression of IFNγ-induced
432 airway epithelial disruption.[51] No difference in expression of *Il10* between strains at
433 chronic time points was observed after DRA administration in our study. However,
434 temporal changes in IL-10 in C57BL/6 mice may contribute to suppression of IFNγ
435 alongside IL-17A.
436
437 IL-13 production is known to be higher in BALB/c versus C57BL/6 mice[26], as also shown
438 here, and is thought to account for increased AHR observed in BALB/c compared to the
439 relatively hypo-responsive C57BL/6 mice.[22,52] In fact, type 2 cytokine producing cells,
440 rather than eosinophilic inflammation, appear to be key for the maintenance of AHR in
441 models of type 2 airway inflammation.[53,23] In addition to enhanced type 2 cytokine
442 production, pathway analysis suggested a reduced capacity (in C57BL/6 mice) for
443 antigen-presenting cell (APC) mediated activation of T cells via costimulatory molecules
444 PD-1/PDL-1 and OX40/OX40L, despite enhanced 'DC maturation' pathways from
445 NanoString analysis in these mice. Different DC subsets can dictate the allergic immune
446 response and targeting either DC activation or molecules involved in antigen
447 presentation may be a fruitful approach to therapeutically target allergic asthma, and
448 specifically different immune phenotypes of disease.[54,55] PDL1 is known to enhance AHR
449 and Th2 cytokine production in allergic mice.[56] Thereby a reduction in PD1-PDL1
450 signalling, alongside reduced OX40 signalling may explain the reduced Th2 response in
451 C57BL/6 mice compared to BALB/c. Future research identifying specific dendritic cell
452 phenotypes in both strains during AAI could prove useful for understanding pathways to
453 target AHR and inflammation in asthma.
454
455 In this model of Th2/Th17 allergic airway inflammation, both BALB/c and C57BL/6 mice
456 developed a similar degree of airway remodelling in response to allergen exposure.
457 However, our study reveals intriguing differences in ECM composition between mouse

strains, not only in response to allergens but also in the steady state. One could anticipate that changes in collagen composition, particularly centred around the ratios of collagen I and III, could profoundly alter lung function and along with varied immune responses, may contribute to well reported differences in AHR measurement between mouse strains.[22,52] Both collagen I and III play major roles in the structural integrity of tissues and are often co-expressed within the tissue, with Col I contributing to tensile strength, whilst Col III allows tissue flexibility.[57] Collagen III can modulate scar formation[58] and during early active fibrosis levels of Col III significantly increase.[59,60] However, an increased ratio between type I and type III collagen occurs in infants diagnosed with chronic lung disease proceeding respiratory distress syndrome.[59] Additionally, a lack of collagen III can disturb the development of collagen fibril formation resulting in functional failure of the organ.[61,62] Here, allergic BALB/c mice appeared to have preferential increase in collagen I around the airways, with no significant changes to collagen III, although we cannot rule out expression of collagen III at time points earlier than week 4. A failure to induce collagen III during remodelling processes may in fact perturb lung function, perhaps contributing to increases in AHR often observed in BALB/c mice in response to allergen challenge.[22,26,52] Differential dynamics in col IV expression between mouse strains is also intriguing, as Col IV is crucial for barrier formation anchoring airway epithelial cells. The rapid loss of Col IV in BALB/c mice may relate to a significant increased vimentin-positive cells around the airway at week 4, and potentially enhanced epithelial mesenchymal transition leading to a more rapid remodelling response in BALB/c versus C57BL/6. Although both mouse strains feature a similar magnitude of allergen-induced remodelling, further analysis of the early dynamics, pre-week 4, and mechanisms leading to changes in the ECM in these two mouse strains may reveal important features of tissue remodelling in disease.

Remodelling is typically examined as a change in epithelial goblet hyperplasia, increased muscle mass and total collagen, but here our study in genetically distinct mouse strains, highlights that the term remodelling is much more complicated. Just as inflammation varies greatly between asthmatic cohorts, airway remodelling too may be considered an "umbrella" term, whereby different pathways are likely to be more or less important in different asthma phenotypes. A greater understanding of how ECM composition changes

490 can alter lung mechanics/function, but also how the differing ECM components can
491 regulate immune cell recruitment and activation, will help us to understand the
492 development of lung diseases like asthma and whether approaches to target remodelling
493 will prove useful in treating such chronic inflammatory diseases. Furthermore, it is
494 interesting to speculate that different genetic strains of mice, rather than using different
495 allergens of timings of allergen exposure, could prove more useful for modelling different
496 trajectories of allergic asthma in people.
497

498 ## Methods

499 ### Animals and Ethics

500 Wild-type (BALB/c or C57BL?6J) mice were obtained from a commercial supplier
501 (Envigo, Hillcrest, UK). Experimental mice, all female, were between 7-10 weeks old at
502 the start of the experiment and were housed in individually ventilated cages maintained
503 in groups of 5 animals in specific pathogen-free facilities at the University of Manchester.
504 Mice were not randomised in cages, but each cage was randomly assigned to a treatment
505 group. Sample size was calculated on the basis of the number of animals needed for
506 detection of a 25% change in Masson's trichrome positive area around the airway in PBS
507 versus allergic mice, with a P value of <0.05, based on pilot experiments carried out
508 with 3 mice per group. All animal experiments were performed in accordance with the
509 UK Animals (Scientific Procedures) Act of 1986 under a Project License (70/8548)
510 granted by the UK Home Office and approved by the University of Manchester Animal
511 Welfare and Ethical Review Body. Euthanasia was performed by asphyxiation in a rising
512 concentration of carbon dioxide.

513

514 ### Model of allergic airway inflammation

515 Allergic airway inflammation was induced in mice in a similar manner as has been
516 described previously.[27] Allergen DRA cocktail comprising of 5 µg House Dust Mite
517 (*Dermatophagoides pteronyssinus,* 5450 EU, 69.23 mg per vial), 50 µg Ragweed
518 (*Ambrosia artemisifolia*), 5 µg *Aspergillus fumigatus* extracts (Greer Laboratories, Lenoir,
519 NC, USA) were freshly prepared prior to each inoculation. Mice were briefly
520 anaesthetised via inhalation of isoflurane, and 20 µL of DRA cocktail or PBS were given
521 via intranasal inoculation twice weekly for up to 8 weeks. Mice were rested for 5 days
522 prior to performing BAL and collecting lung tissue.

523

524 ### Isolation of cells from the BAL and lung tissue

525 Following exsanguination, BAL cells were obtained through cannulation of the trachea
526 and washing the lungs with 0.4 mL PBS (Sigma Aldrich, St. Louis, MO, USA) containing
527 0.25 % BSA (Sigma Aldrich) (four washes). Lungs were processed as previously
528 described.[40] Briefly, a right lobe was removed and minced in 1 mL of HBSS buffer

529 containing 0.4 U mL^{-1} Liberase TL (Sigma Aldrich) and 80 U mL^{-1} DNase type I
530 (ThermoFisher Scientific, Waltham, MA, USA) for 25 min in a 37ºC shaking incubator.
531 Digestion was stopped with 2 % FBS (ThermoFisher Scientific) and 2 mM EDTA prior to
532 passing the suspension through a 70 µm cell strainer (Greiner Bio-One, Stonehouse, UK).
533 Red blood cells were lysed (Sigma) and total live BAL and lung cell counts assessed with
534 Viastain AOPI (Nexcelom Bioscience LLC, Lawrence, MA, USA) using a Cellometer
535 Auto2000 automated cell counter (Nexcelom Bioscience LLC).
536
537 **Flow Cytometry**
538 Equal cell numbers of each lung and BAL sample were stained for flow cytometry. Cells
539 were washed with ice-cold PBS and stained with Live/Dead Aqua or Blue (ThermoFisher
540 Scientific) for 10 min at room temperature. All samples were then incubated with Fc
541 block (5 µg mL^{-1} CD16/CD32 (BD Biosciences, San Diego, CA, USA) and 0.1 % mouse
542 serum in FACs buffer (PBS containing 0.5 % BSA and 2 mM EDTA (ThermoFisher
543 Scientific)) for 20 min before staining for specific surface markers with fluorescence-
544 conjugated antibodies for 25 min at 4ºC (**Table 1**) Following surface staining, cells were
545 fixed with ICC fix (Biolegend, San Diego, CA, USA) and stored at 4ºC until intracellular
546 staining was performed or cells were acquired. For intracellular cytokine staining, cells
547 were stimulated for 4h at 37ºC with PMA (phorbol myristate acetate; 0.5 µg mL^{-1}; Sigma
548 Aldrich) and ionomycin (1 µg mL^{-1})(Sigma Aldrich) and for 3 h at 37ºC with Brefaldin A
549 (10 µg mL^{-1}; Biolegend). Cell surfaces were stained and cells fixed as described above.
550 All cells were permeabilized (eBioscience, San Diego, CA, USA) then stained with
551 antibodies for intracellular cytokines (**Table 1**). Cells were identified with the following
552 markers: eosinophils F4/80$^+$ CD11c$^-$ CD11b$^+$ SigF$^+$; neutrophils Ly6G$^+$ CD11b$^+$ CD11c$^-$;
553 T cells TCRβ$^+$ TCRɣδ$^-$ and either CD4$^+$ or CD8$^+$; gamma delta T cells TCRβ$^-$ TCRɣδ$^+$ CD4$^-$
554 CD8$^-$; innate lymphoid cells (ILCs) CD90$^+$ ICOS$^+$ Lineage$^-$ (CD11b, Ly6G, Ly6C, CD11c,
555 Ter119, NK1.1, B220, CD3). All samples were acquired with a FACS Canto II or 5 laser
556 Fortessa with BD FACS Diva software and analysed with FlowJo software (versions 9 and
557 10; BD Biosciences).
558
559

560 **RNA extraction and qRT-PCR**

561 One right lung lobe was stored in RNAlater (ThermoFisher Scientific) prior to
562 homogenization in Qiazol reagent (Qiagen, Hilden, Germany). RNA was prepared
563 according to manufacturer's instructions and stored at -70 ºC. Reverse transcription of
564 0.2-0.5 µg total RNA was performed using 50 U Tetro reverse transcriptase (Bioline,
565 London, UK), 40 mM dNTPs (Promega), 0.5 µg primer for cDNA synthesis (Sigma
566 Aldrich) and RNasin inhibitor (Promega, Madison, WI, USA). The transcripts for genes of
567 interest were measured by real-time PCR with a Lightcycler 480 II system (Roche, Basel,
568 Switzerland) and a Brilliant III SYBR Green Master mix (Agilent Technologies, Santa
569 Clara, CA, USA) with specific primer pairs (**Table 2**). mRNA amplification was analysed
570 by second derivative maximum algorithm (LightCycler 480 Sw 1.5; Roche) and
571 expression of the gene of interest was normalised to the geometric mean of three
572 housekeeping genes *Rn45s*, *Rpl13a*, *Gapdh* (**Table 2**).

573

574 **Transcriptome profile and associated analysis**

575 Quality of RNA extracted from lung tissue, as described above, was assessed with Agilent
576 2200 TapeStation system prior to downstream analyses, samples with an RIN value of
577 <5.5 were excluded. RNA concentration was determined using Qubit TM RNA BR Assay
578 Kit (ThermoFisher Scientific) and 100 ng RNA (per sample) run on a Nanotstring
579 nCounter R FLEC system using the Myeloid Innate Immunity v2 panel (XT-CSO-MMII2-
580 12). Note, the probes in this panel do not distinguish between *Chil3* and *Chil4*. Raw
581 counts were uploaded onto nSolver version 4.0 using default settings. Non-normalised
582 counts were exported, and subsequent analyses performed in R (version 3.6.3) using
583 RStudio Version 1.2.5033 (2009-2019 RStudio, Inc, Boston, MA, USA). Positive controls
584 were analysed to ensure there was clear resolution at variable expression levels and
585 negative controls were used to set a minimum detection threshold which was then
586 applied to all samples. Data were normalised with EdgeR using the Upper Quartile
587 method and differential expression of genes calculated via linear modelling accounting
588 for sample quality weights with Empirical Bayes smoothing using the limma-voom R
589 packages.[63] All genes expressed above the background threshold were used for principal
590 component analysis (PCA). Genes with an absolute fold change of greater than 0.5 and
591 a significance value of under 0.05 after correction for multiple comparisons using the

592 Benjamini-Yekeuteli method were defined as "differentially expressed" and taken
593 forward for further analysis. Heatmaps were then generated from scaled normalized
594 counts of DE genes using the ComplexHeatmaps R package. The networks and functional
595 analyses of DE genes were generated with Ingenuity Pathway Analyser (IPA; QIAGEN
596 Inc., https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis).
597 Within the IPA software no tissue filtering was used and the user dataset was defined as
598 the reference. Pathway data were then imported into R for visualisation using the ggplot
599 package.
600
601 **Generating Anti-Ym2 and determining antibody specificity**
602 Anti-Ym2 specific antibodies were generated by Cambridge Research Biochemicals
603 (Billingham, UK). The 9 amino acid sequence at the N-terminal (CKASYRGEL) were used
604 as the immunogen as it has almost no homology to the Ym1 sequence. Bacterial
605 optimised expression plasmids for Ym1 and Ym2 were purchased from (Genscript,
606 Piscataway, NJ, USA). Plasmids were then transfected into competent *E. Coli* (BL21) using
607 heat shock followed antibiotic selection against Ampicillin (Amp; 25 mg mL^{-1}) and
608 Chloramphenicol (Chl; 34 mg mL^{-1}). To generate recombinant protein a small scraping
609 of the stock sample was expanded in LB media including antibiotics until optical density
610 reached between 0.6-1.0 at which point IPTG (0.1 M) was added to the cultures. The
611 OD was kept under 1.0 by diluting the culture with fresh media as required and left
612 overnight. Thereafter bacteria were pelleted and resuspended in loading buffer
613 containing DTT (200 mM; ThermoFischer Scientific).
614
615 **Western Blotting**
616 Lysed Ym1 or Ym2 transfected *E.coli* cells and murine BAL were denatured in the
617 presence of DTT (200 mM; Thermo Fischer Scientific) for 5 mins at 95ºC. Each sample
618 (2-10 µL) or protein ladder (Seeblue; ThermoFischer Scientific) was separated on Bis-Tris
619 4-12 % gradient gel with MES buffer (ThermoFischer Scientific) before transfer onto a
620 PVDF membrane. The membrane was washed in distilled water followed by incubation
621 in blocking buffer (5 % BSA in PBST (0.05 % Tween-20 in PBS)) for 60 mins at room
622 temperature on a rocking platform. Primary antibodies were used at 1:500 (rabbit anti-
623 mouse Ym2, polyclonal (custom made) or goat anti-mouse Ym1 polyclonal; R&D

624 Systems, Minneapolis, MN, USA) and incubated at room temperature over-night on a
625 rocking platform. The membrane was then washed in PBST followed by secondary
626 antibody detection (1:1000 anti-Rabbit IgG Cy3 and Streptavidin-Cy3; ThermoFisher
627 Scientific) for one hour at room temperature. Membranes were imaging using a Gel Doc
628 (Azure Biosystems, Cambridge Bioscience, Cambridge, UK).
629
630 **Histology and Immunostaining**
631 The left lung lobe was fixed perfused with 10 % neutral buffered formalin (Sigma Aldrich)
632 and was incubated overnight before being transferred to 70 % ethanol. Lungs were
633 processed and embedded in paraffin, then sectioned (5 μm) and stained with Masson's
634 trichrome (MT) or periodic acid schiff (PAS) stains using standard protocols. Images were
635 captured with a Leica microscope with digital DMC2900 camera. For immunostaining
636 with antibodies, lung sections were deparaffinised and heat-mediated antigen retrieval
637 performed using Tris-EDTA buffer (10 mM Tris Base, 1 mM EDTA, 0.05 % Tween-20 pH
638 8.0; incubation 20 min 95ºC). Non-specific protein was blocked with 2 % normal
639 donkey serum (Sigma Aldrich) in PBS containing 0.05 % Tween-20 and 1 % BSA. If a
640 biotin labelled antibody or probe was used, avidin biotin block (ThermoFisher Scientific)
641 was performed prior to an overnight incubation at 4ºC with primary antibodies (Table 3).
642 Sections were washed in PBS before incubation with secondary antibodies (**Table 3**) for
643 1 hr at room temperature followed by mounting with DAPI containing fluoromount
644 (Southern Biotech, Birmingham, AL, USA). Images were captured with an EVOS FL
645 imaging system (ThermoFisher Scientific). Analysis of images was performed using
646 ImageJ software (version 2.09.0-rc69/1.52p) on sections where sample identification was
647 blinded for the investigator and airways analysed had to be intact and fit within a single
648 microscope field of view 480 μm x 360 μm. Goblet cells were visualised on PAS-stained
649 sections and numbers of PAS+ cells counted per airway and normalised to the length the
650 airway basement membrane. Total collagen area was calculated by measuring the area
651 of Masson's trichrome positive stain (blue) around the airway and values were normalised
652 to basement membrane length. For calculation of collagen, hyaluronan, vimentin and
653 αSMA area, background autofluorescence was subtracted from all images based on pixel
654 intensities of sections stained with secondary antibodies only. A region of interest was
655 drawn parallel to the airway basement membrane at a distance of 50 μm. A threshold

656 was applied to all images to incorporate positively stained pixels and area of positively
657 stained pixels within the region of interest calculated and normalised to the length of the
658 basement membrane. All areas of the airway that contained a blood vessel was excluded
659 from analysis to ensure measurements specifically related to airways and not vasculature.
660 For all image analysis, between 5-15 airways were measured per mouse.
661
662 **Quantification of Ym1 and BRP-39**
663 The levels of Ym1 and BRP-39 in the serum and BAL were measured by sandwich ELISA,
664 with DuoSet ELISA kits (R&D Systems) as per manufacturers recommendation.
665
666 **Statistical analysis**
667 Statistical analysis was performed using JMP Pro 12.2.0 for Mac OS X (SAS Institute Inc.,
668 Cary, NC, USA). Normal distribution of data was determined by optical examination of
669 residuals, and each group was tested for unequal variances using Welch's test.
670 Differences between groups were determined by analysis of variance (ANOVA) followed
671 by a Tukey-Kramer HSD multiple comparison test or unpaired two-tailed Student's t-test
672 as indicated in figure legends. In some data sets, data were log transformed to achieve
673 normal distribution. Differences were considered statistically significant for *P* values of
674 less than 0.05.

Acknowledgments

This work was supported by the Medical Research Foundation UK jointly with Asthma UK (MRFAUK-2015-302 to TES), the Medical Research Council UK (MR/P02615X/1 to DR and MR/K01207X/1 to JEA) and the Wellcome Trust (106898/A/15/Z to JEA). The authors would like to thank Brian Chan for his technical support, Alistair Chenery and Anthony Day for *E. coli* expressing recombinant Ym1 and Ym2 and Conor Finlay for critical reading of the manuscript. We also thank the Flow Cytometry, Histology and Biological Services core facilities at the University of Manchester.

Conflict of Interest

The authors declare no competing financial interests.

Author Contributions

JEP: Data curation; investigation; methodology; formal analysis; writing – original draft; Writing – reviewing and editing. **SP**: investigation; methodology. **DR:** investigation; writing – reviewing and editing. **JEA**: Funding acquisition; investigation; writing – reviewing and editing. **TES**: Conceptualisation; Investigation; methodology; project administration; supervision; funding acquisition; writing – original draft; writing – review and editing.

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Table 1: Antibodies used for flow cytometry analysis

Antigen	Antibody Clone	Isotype	Source
Ly6G	1A8	Rat IgG2a κ	Biolegend
CD11b	M1/70	Rat IgG2b κ	Biolegend
CD11c	N418	Armenian Hamster IgG	Biolegend
F4/80	BM8	Rat IgG2a κ	Biolegend
CD64	X54-5/7.1	Mouse IgG1 κ	Biolegend
SiglecF	E50-2440	Rat IgG2a κ	BD Biosciences
I-A/I-E	M5/144.15.2	Rat IgG2b κ	Biolegend
TCRβ	H57-597	Armenian Hamster IgG	eBioscience
TCRγδ	GL3	Armenian Hamster IgG	Biolegend
CD4	GK1.5	Rat IgG2b κ	Biolegend
CD8	53-6.7	Rat IgG2a κ	Biolegend
CD3	17A2	Rat IgG2b κ	Biolegend
B220	RA3-682	Rat IgG2a κ	Biolegend
Ter119	Ter-119	Rat IgG2b κ	Biolegend
NK1.1	PK136	Mouse IgG2a κ	Biolegend
ICOS	C398.4A	Armenian Hamster IgG	Biolegend
CD90.2	30-H12	Rat IgG2b κ	Biolegend
IL-4	11b11	Rat IgG1 κ	Biolegend
IL-13	eBio13A	Rat IgG1 κ	eBioscience
IL-17a	TC11-18H10.1	Rat IgG1 κ	Biolegend
IFNγ	XMG1.2	Rat IgG1 κ	Biolegend

Table 2: Sequences of primers for measurement of mRNA expression via quantitative RT-PCR

Gene	Forward Primer	Reverse Primer
Il4	CCTGCTCTTCTTTCTCGAATG	CACATCCATCTCCGTGCAT
Il13	CCTCTGACCCTTAAGGAGCTTAT	CGTTGCACAGGGGAGTCT
Il5	ACATTGACCGCCAAAAAGAG	CACCATGGAGCAGCTCAG
Il17a	GCTCCAGAAGGCCCTCAGACT	CCAGCTTTCCCTCCGCATTGA
Ifng	GGAGGAACTGGCAAAAGGAT	TTCAAGACTTCAAAGAGTCTGAGG
Chil1	CCAGCCAGGCAGAGAGAAAC	GCCACCTTTCCTGCTGACA
Chil3	TCTGGTGAAGGAAATGCGTAAA	GCAGCCTTGGAATGTCTTTCTC
Chil4	TCTGGTGCAGGAAATGCGTAAA	GCAGCCTTGGAATGTGGTTCAAAG
Rn45s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Rpl13a	CATGAGGTCGGGTGGAAGTA	GCCTGTTTCCGTAACCTCAA
Gapdh	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG

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916 **Table 3: Antibodies used for immuno-histological analysis**

Antigen	Antibody Clone	Dilution	Source
Ym1	Goat polyclonal - Biotinylated	1:100	R&D – BAF2446
Ym2	Rabbit polyclonal	1:1000	Home-made
Collagen I	Goat polyclonal	1:200	Cambridge Bioscience – 1310-01
Collagen III (N-terminal)	Rabbit polyclonal	1:300	Proteintech – 22734-1-AP
Collagen IV alpha I	Rabbit polyclonal	1:200	Novus Biologicals – NB120-6586
Hyaluronan binding protein	Biotinylated	1:100	Merck Millipore - 385911
α-Smooth muscle actin	Goat polyclonal	1:200	Novus Biologicals – NB-300-978
Vimentin	Rabbit polyclonal	1:200	Abcam – ab45939
BRP-39	Rabbit Polyclonal	1:100	Biorbyt – orb10365
-	Streptavidin 557	1:800	R&D – NL999
-	Streptavidin 637	1:400	R&D – NL998
-	Donkey anti-rabbit IgG 557	1:200	R&D – NL004
-	Donkey anti-rabbit IgG 637	1:200	R&D – NL005
-	Donkey anti-goat IgG 557	1:200	R&D – NL001
-	Donkey anti-goat IgG 637	1:200	R&D – NL002

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